methods may be used in conjunction with known cancer therapeutics to screen for agonists to make the therapeutic more effective or less toxic. This is particularly preferred when the chemotherapeutic is very expensive to produce such as taxol.

[0834] Known oncogenes such as v-Abl, v-Src, v-Ras, and others, induce a transformed phenotype leading to abnormal cell growth when transfected into certain cells. This is also a major problem with micro-metastases. Thus, in a preferred embodiment, non-transformed cells can be transfected with these oncogenes, and then libraries introduced into these cells, to select for target polynucleotides which reverse or correct the transformed state. One of the signal features of oncogene transformation of cells is the loss of contact inhibition and the ability to grow in soft-agar. When transforming viruses are constructed containing v-Abl, v-Src, or v-Ras in viral vectors, infected into target 3T3 cells, and subjected to puromycin selection, all of the 3T3 cells hyper-transform and detach from the plate. The cells may be removed by washing with fresh medium. This can serve as the basis of a screen, since cells which express a target polynucleotides will remain attached to the plate and form colonies.

[0835] Similarly, the growth and/or spread of certain tumor types is enhanced by stimulatory responses from growth factors and cytokines (PDGF, EGF, Heregulin, and others) which bind to receptors on the surfaces of specific tumors. In a preferred embodiment, the methods of the invention are used to inhibit or stop tumor growth and/or spread, by finding target polynucleotides capable of blocking the ability of the growth factor or cytokine to stimulate the tumor cell. The introduction of libraries into specific tumor cells with the addition of the growth factor or cytokine, followed by selection of target polynucleotides which block the binding, signaling, phenotypic and/or functional responses of these tumor cells to the growth factor or cytokine in question.

[0836] Similarly, the spread of cancer cells (invasion and metastasis) is a significant problem limiting the success of cancer therapies. The ability to inhibit the invasion and/or migration of specific tumor cells would be a significant advance in the therapy of cancer. Tumor cells known to have a high metastatic potential (for

example, melanoma, lung cell carcinoma, breast and ovarian carcinoma) can have libraries introduced into them, and target polynucleotides selected which in a migration or invasion assay, inhibit the migration and/or invasion of specific tumor cells. Particular applications for inhibition of the metastatic phenotype, which could allow a more specific inhibition of metastasis, include the metastasis suppressor gene NM23, which codes for a dinucleoside diphosphate kinase. Thus intracellular peptide activators of this gene could block metastasis, and a screen for its upregulation (by fusing it to a reporter gene (e.g. indicator gene)) would be of interest. Many oncogenes also enhance metastasis. Peptides which inactivate or counteract mutated RAS oncogenes, v-MOS, v-RAF, A-RAF, v-SRC, v-FES, and v-FMS would also act as anti-metastatics. Target polynucleotides which block the release of combinations of proteases required for invasion, such as the matrix metalloproteases and urokinase, could also be effective antimetastatics.

[0837] In a preferred embodiment, the libraries of the present invention are introduced into tumor cells known to have inactivated tumor suppressor genes, and successful reversal by either reactivation or compensation of the knockout would be screened by restoration of the normal phenotype. A major example is the reversal of p53-inactivating mutations, which are present in 50% or more of all cancers. Since p53's actions are complex and involve its action as a transcription factor, there are probably numerous potential ways a target polynucleotides could reverse the mutation. One example would be upregulation of the immediately downstream cyclin-dependent kinase p21CIP1/WAF1. To be useful such reversal would have to work for many of the different known p53 mutations. This is currently being approached by gene therapy; one or more small molecules which do this might be preferable.

[0838] Another example involves screening of target polynucleotides which restore the constitutive function of the brca-1 or brca-2 genes, and other tumor suppressor genes important in breast cancer such as the adenomatous polyposis coli gene (APC) and the Drosophila discs-large gene (Dlg), which are components of cell--

cell junctions. Mutations of brca-1 are important in hereditary ovarian and breast cancers, and constitute an additional application of the present invention.

[0839] In a preferred embodiment, the methods of the present invention are used to create novel cell lines from cancers from patients. A target polynucleotide which inhibits the final common pathway of programmed cell death should allow for short- and possibly long-term cell lines to be established. Conditions of in vitro culture and infection of human leukemia cells will be established. There is a real need for methods which allow the maintenance of certain tumor cells in culture long enough to allow for physiological and pharmacological studies. Currently, some human cell lines have been established by the use of transforming agents such as Epstein-Barr virus that considerably alters the existing physiology of the cell. On occasion, cells will grow on their own in culture but this is a random event. Programmed cell death (apoptosis) occurs via complex signaling pathways within cells that ultimately activate a final common pathway producing characteristic changes in the cell leading to a non-inflammatory destruction of the cell. It is well known that tumor cells have a high apoptotic index, or propensity to enter apoptosis in vivo. When cells are placed in culture, the in vivo stimuli for malignant cell growth are removed and cells readily undergo apoptosis. The objective would be to develop the technology to establish cell lines from any number of primary tumor cells, for example primary human leukemia cells, in a reproducible manner without altering the native configuration of the signaling pathways in these cells. By introducing target polynucleotides which inhibit apoptosis, increased cell survival in vitro, and hence the opportunity to study signalling transduction pathways in primary human tumor cells, is accomplished. In addition, these methods may be used for culturing primary cells, i.e. non-tumor cells.

[0840] In a preferred embodiment, the present methods are useful in cardiovascular applications. In a preferred embodiment, cardiomyocytes may be screened for the prevention of cell damage or death in the presence of normally injurious conditions, including, but not limited to, the presence of toxic drugs (particularly chemotherapeutic drugs), for example, to prevent heart failure following treatment

with adriamycin; anoxia, for example in the setting of coronary artery occlusion; and autoimmune cellular damage by attack from activated lymphoid cells (for example as seen in post viral myocarditis and lupus). Insert polynucleotides are introduced into cardiomyocytes, the cells are subjected to the insult, and target polynucleotides are selected that prevent any or all of: apoptosis; membrane depolarization (i.e. decrease arrythmogenic potential of insult); cell swelling; or leakage of specific intracellular ions, second messengers and activating molecules (for example, arachidonic acid and/or lysophosphatidic acid).

[0841] In a preferred embodiment, the present methods are used to screen for diminished arrhythmia potential in cardiomyocytes. The screens comprise the introduction of the insert polynucleotides encoding candidate target polynucleotides, followed by the application of arrythmogenic insults, with screening for target polynucleotides that block specific depolarization of cell membrane. This may be detected using patch clamps, or via fluorescence techniques). Similarly, channel activity (for example, potassium and chloride channels) in cardiomyocytes could be regulated using the present methods in order to enhance contractility and prevent or diminish arrhythmias.

[0842] In a preferred embodiment, the present methods are used to screen for enhanced contractile properties of cardiomyocytes and diminish heart failure potential. The introduction of the libraries of the invention followed by measuring the rate of change of myosin polymerization/depolymerization using fluorescent techniques can be done. Target polynucleotides which increase the rate of change of this phenomenon can result in a greater contractile response of the entire myocardium, similar to the effect seen with digitalis.

[0843] In a preferred embodiment, the present methods are useful to identify target polynucleotides that will regulate the intracellular and sarcolemmal calcium cycling in cardiomyocytes in order to prevent arrhythmias. Target polynucleotides are selected that regulate sodium-calcium exchange, sodium proton pump function, and regulation of calcium-ATPase activity.

[0844] In a preferred embodiment, the present methods are useful to identify molecules that diminish embolic phenomena in arteries and arterioles leading to strokes (and other occlusive events leading to kidney failure and limb ischemia) and angina precipitating a myocardial infarct are selected. For example, target polynucleotides which will diminish the adhesion of platelets and leukocytes, and thus diminish the occlusion events. Adhesion in this setting can be inhibited by the libraries of the invention being inserted into endothelial cells (quiescent cells, or activated by cytokines, i.e. IL-1, and growth factors, i.e. PDGF / EGF) and then screening for target polynucleotides that either: 1) downregulate adhesion molecule expression on the surface of the endothelial cells (binding assay); 2) block adhesion molecule activation on the surface of these cells (signaling assay); or 3) release in an autocrine manner peptides that block receptor binding to the cognate receptor on the adhering cell.

[0845] Embolic phenomena can also be addressed by activating proteolytic enzymes on the cell surfaces of endothelial cells, and thus releasing active enzyme which can digest blood clots. Thus, delivery of the libraries of the invention to endothelial cells is done, followed by standard fluorogenic assays, which will allow monitoring of proteolytic activity on the cell surface towards a known substrate. Target polynucleotides can then be selected which activate specific enzymes towards specific substrates.

[0846] In a preferred embodiment, arterial inflammation in the setting of vasculitis and post-infarction can be regulated by decreasing the chemotactic responses of leukocytes and mononuclear leukocytes. This can be accomplished by blocking chemotactic receptors and their responding pathways on these cells. Libraries can be inserted into these cells, and the chemotactic response to diverse chemokines (for example, to the IL-8 family of chemokines, RANTES) is inhibited in cell migration assays.

[0847] In a preferred embodiment, arterial restenosis following coronary angioplasty can be controlled by regulating the proliferation of vascular intimal cells and capillary and/or arterial endothelial cells. Libraries can be inserted into these

cell types and proliferation in response to specific stimuli is monitored. One application may be target polynucleotides which block the expression or function of c-myc and other oncogenes in smooth muscle cells to stop their proliferation. A second application may involve the expression of libraries in vascular smooth muscle cells to selectively induce their apoptosis. Application of therapeutics derived from these target polynucleotides require targeted drug delivery; this is available with stents, hydrogel coatings, and infusion-based catheter systems. Target polynucleotides which downregulate endothelin-1A receptors or which block the release of the potent vasoconstrictor and vascular smooth muscle cell mitogen endothelin-1 may also be candidates for therapeutics. Target polynucleotides can be isolated from these libraries which inhibit growth of these cells, or which prevent the adhesion of other cells in the circulation known to release autocrine growth factors, such as platelets (PDGF) and mononuclear leukocytes.

[0848] The control of capillary and blood vessel growth is an important goal in order to promote increased blood flow to ischemic areas (growth), or to cut-off the blood supply (angiogenesis inhibition) of tumors. Libraries can be inserted into capillary endothelial cells and their growth monitored. Stimuli such as low oxygen tension and varying degrees of angiogenic factors can regulate the responses, and target polynucleotides isolated that produce the appropriate phenotype. Screening for antagonism of vascular endothelial cell growth factor, important in angiogenesis, would also be useful.

[0849] In a preferred embodiment, the present methods are useful in screening for decreases in atherosclerosis producing mechanisms to find target polynucleotides that regulate LDL and HDL metabolism. Libraries can be inserted into the appropriate cells (including hepatocytes, mononuclear leukocytes, endothelial cells) and target polynucleotides selected which lead to a decreased release of LDL or diminished synthesis of LDL, or conversely to an increased release of HDL or enhanced synthesis of HDL. Target polynucleotides can also be isolated from libraries which decrease the production of oxidized LDL, which has been

implicated in atherosclerosis and isolated from atherosclerotic lesions. This could occur by decreasing its expression, activating reducing systems or enzymes, or blocking the activity or production of enzymes implicated in production of oxidized LDL, such as 15-lipoxygenase in macrophages.

[0850] In a preferred embodiment, the present methods are used in screens to regulate obesity via the control of food intake mechanisms or diminishing the responses of receptor signaling pathways that regulate metabolism. Target polynucleotides that regulate or inhibit the responses of neuropeptide Y (NPY), cholecystokinin and galanin receptors, are particularly desirable. Libraries can be inserted into cells that have these receptors cloned into them, and inhibitory target polynucleotides selected that are secreted in an autocrine manner that block the signaling responses to galanin and NPY. In a similar manner, target polynucleotides can be found that regulate the leptin receptor.

[0851] In a preferred embodiment, the present methods are useful in neurobiology applications. Libraries may be used for screening for anti-apoptotics for preservation of neuronal function and prevention of neuronal death. Initial screens would be done in cell culture. One application would include prevention of neuronal death, by apoptosis, in cerebral ischemia resulting from stroke. Apoptosis is known to be blocked by neuronal apoptosis inhibitory protein (NAIP); screens for its upregulation, or effecting any coupled step could yield peptides which selectively block neuronal apoptosis. Other applications include neurodegenerative diseases such as Alzheimer's disease and Huntington's disease.

[0852] In a preferred embodiment, the present methods are useful in bone biology applications. Osteoclasts are known to play a key role in bone remodeling by breaking down "old" bone, so that osteoblasts can lay down "new" bone. In osteoporosis one has an imbalance of this process. Osteoclast overactivity can be regulated by inserting libraries into these cells, and then looking for target polynucleotides that produce: 1) a diminished processing of collagen by these cells; 2) decreased pit formation on bone chips; and 3) decreased release of calcium from bone fragments.

[0853] The present methods may also be used to screen for agonists of bone morphogenic proteins, hormone mimetics to stimulate, regulate, or enhance new bone formation (in a manner similar to parathyroid hormone and calcitonin, for example). These have use in osteoporosis, for poorly healing fractures, and to accelerate the rate of healing of new fractures. Furthermore, cell lines of connective tissue origin can be treated with candidate libraries and screened for their growth, proliferation, collagen stimulating activity, and/or proline incorporating ability on the target osteoblasts. Alternatively, libraries can be expressed directly in osteoblasts or chondrocytes and screened for increased production of collagen or bone.

[0854] In a preferred embodiment, the present methods are useful in skin biology applications. Keratinocyte responses to a variety of stimuli may result in psoriasis, a proliferative change in these cells. Libraries can be inserted into cells removed from active psoriatic plaques, and target polynucleotides isolated which decrease the rate of growth of these cells.

[0855] In a preferred embodiment, the present methods are useful in the regulation or inhibition of keloid formation (i.e. excessive scarring). Libraries are inserted into skin connective tissue cells isolated from individuals with this condition, and target polynucleotides are isolated that decrease proliferation, collagen formation, or proline incorporation. Results from this work can be extended to treat the excessive scarring that also occurs in burn patients. If a common peptide motif is found in the context of the keloid work, then it can be used widely in a topical manner to diminish scarring post burn.

[0856] Similarly, wound healing for diabetic ulcers and other chronic "failure to heal" conditions in the skin and extremities can be regulated by providing additional growth signals to cells which populate the skin and dermal layers. Growth factor mimetics may in fact be very useful for this condition. Libraries can be inserted into skin connective tissue cells, and target polynucleotides isolated which promote the growth of these cells under "harsh" conditions, such as low oxygen tension, low pH, and the presence of inflammatory mediators.

[0857] Cosmeceutical applications of the present invention include the control of melanin production in skin melanocytes. A naturally occurring peptide, arbutin, is a tyrosine hydroxylase inhibitor, a key enzyme in the synthesis of melanin. Libraries can be inserted into melanocytes and known stimuli that increase the synthesis of melanin is applied to the cells. Target polynucleotides can be isolated that inhibit the synthesis of melanin under these conditions.

[0858] In a preferred embodiment, the present methods are useful in endocrinology applications. The library technology can be applied broadly to any endocrine, growth factor, cytokine or chemokine network which involves a signaling peptide or protein that acts in either an endocrine, paracrine or autocrine manner that binds or dimerizes a receptor and activates a signaling cascade that results in a known phenotypic or functional outcome. The methods are applied so as to isolate a peptide which either mimics the desired hormone (i.e., insulin, leptin, calcitonin, PDGF, EGF, EPO, GMCSF, IL1-17, mimetics) or inhibits its action by either blocking the release of the hormone, blocking its binding to a specific receptor or carrier protein (for example, CRF binding protein), or inhibiting the intracellular responses of the specific cells to that hormone. Selection of target polynucleotides which increase the expression or release of hormones from the cells which normally produce them could have broad applications to conditions of hormonal deficiency.

[0859] In a preferred embodiment, the present methods are useful in infectious disease applications. Viral latency (herpes viruses such as CMV, EBV, HBV, and other viruses such as HIV) and their reactivation are a significant problem, particularly in immunosuppressed patients (patients with AIDS and transplant patients). The ability to block the reactivation and spread of these viruses is an important goal. Cell lines known to harbor or be susceptible to latent viral infection can be infected with the specific virus, and then stimuli applied to these cells which have been shown to lead to reactivation and viral replication. This can be followed by measuring viral titers in the medium and scoring cells for phenotypic changes. Libraries can then be inserted into these cells under the above conditions, and target polynucleotides are isolated which block or diminish the growth and/or release of

the virus. As with chemotherapeutics, these experiments can also be done with drugs which are only partially effective towards this outcome, and target polynucleotides isolated which enhance the virucidal effect of these drugs.

[0860] One example of many is the ability to block HIV-1 infection. HIV-1 requires CD4 and a co-receptor which can be one of several seven transmembrane G-protein coupled receptors. In the case of the infection of macrophages, CCR-5 is the required co-receptor, and there is strong evidence that a block on CCR-5 will result in resistance to HIV-1 infection. There are two lines of evidence for this statement. First, it is known that the natural ligands for CCR-5, the CC chemokines RANTES, MIP1a and MIP1b are responsible for CD8+ mediated resistance to HIV. Second, individuals homozygous for a mutant allele of CCR-5 are completely resistant to HIV infection. Thus, an inhibitor of the CCR-5/HIV interaction would be of enormous interest to both biologists and clinicians. One infects a cell line that expresses CCR-5 with viral vectors containing library. Using an antibody to CCR-5 one can use FACS to sort desired cells based on the binding of this antibody to the receptor. All cells which do not bind the antibody will be assumed contain target polynucleotides which inhibit expression of this antibody binding site. These target polynucleotides can be further assayed for their ability to inhibit HIV-1 entry.

[0861] Viruses are known to enter cells using specific receptors to bind to cells (for example, HIV uses CD4, coronavirus uses CD13, murine leukemia virus uses transport protein, and measles virus usesCD44) and to fuse with cells (HIV uses chemokine receptor). Libraries can be inserted into target cells known to be permissive to these viruses, and target polynucleotides are isolated which block the ability of these viruses to bind and fuse with specific target cells.

[0862] In a preferred embodiment, the present invention finds use with infectious organisms. Intracellular organisms such as mycobacteria, listeria, salmonella, pneumocystis, yersinia, leishmania, T. cruzi, can persist and replicate within cells, and become active in immunosuppressed patients. There are currently drugs on the market and in development which are either only partially effective or ineffective against these organisms. Libraries can be inserted into specific cells infected with

these organisms (pre- or post-infection), and target polynucleotides selected which promote the intracellular destruction of these organisms in a manner analogous to intracellular "antibiotic peptides" similar to magainins. In addition target polynucleotides can be selected which enhance the cidal properties of drugs already under investigation which have insufficient potency by themselves, but when combined with a specific peptide from a candidate library, are dramatically more potent through a synergistic mechanism. Finally, target polynucleotides can be isolated which alter the metabolism of these intracellular organisms, in such a way as to terminate their intracellular life cycle by inhibiting a key organismal event.

[0863] Antibiotic drugs that are widely used have certain dose dependent, tissue specific toxicities. For example renal toxicity is seen with the use of gentamicin, tobramycin, and amphotericin; hepatotoxicity is seen with the use of INH and rifampin; bone marrow toxicity is seen with chloramphenicol; and platelet toxicity is seen with ticarcillin, etc. These toxicities limit their use. Libraries can be introduced into the specific cell types where specific changes leading to cellular damage or apoptosis by the antibiotics are produced, and target polynucleotides can be isolated that confer protection, when these cells are treated with these specific antibiotics.

[0864] Furthermore, the present invention finds use in screening for target polynucleotides that block antibiotic transport mechanisms. The rapid secretion from the blood stream of certain antibiotics limits their usefulness. For example penicillins are rapidly secreted by certain transport mechanisms in the kidney and choroid plexus in the brain. Probenecid is known to block this transport and increase serum and tissue levels. Insert polynucleotides can be introduced into specific cells derived from kidney cells and cells of the choroid plexus known to have active transport mechanisms for antibiotics. Target polynucleotides can then be isolated which block the active transport of specific antibiotics and thus extend the serum half life of these drugs.

[0865] In a preferred embodiment, the present methods are useful in drug toxicities and drug resistance applications. Drug toxicity is a significant clinical problem. This

may manifest itself as specific tissue or cell damage with the result that the drug's effectiveness is limited. Examples include myeloablation in high dose cancer chemotherapy, damage to epithelial cells lining the airway and gut, and hair loss. Specific examples include adriamycin induced cardiomyocyte death, cisplatinin-induced kidney toxicity, vincristine-induced gut motility disorders, and cyclosporin-induced kidney damage. Libraries can be introduced into specific cell types with characteristic drug-induced phenotypic or functional responses, in the presence of the drugs, and target polynucleotides isolated which reverse or protect the specific cell type against the toxic changes when exposed to the drug. These effects may manifest as blocking the drug induced apoptosis of the cell of interest, thus initial screens will be for survival of the cells in the presence of high levels of drugs or combinations of drugs used in combination chemotherapy.

[0866] Drug toxicity may be due to a specific metabolite produced in the liver or kidney which is highly toxic to specific cells, or due to drug interactions in the liver which block or enhance the metabolism of an administered drug. Libraries can be introduced into liver or kidney cells following the exposure of these cells to the drug known to produce the toxic metabolite. Target polynucleotides can be isolated which alter how the liver or kidney cells metabolize the drug, and specific molecules identified which prevent the generation of a specific toxic metabolite. The generation of the metabolite can be followed by mass spectrometry, and phenotypic changes can be assessed by microscopy. Such a screen can also be done in cultured hepatocytes, cocultured with readout cells which are specifically sensitive to the toxic metabolite. Applications include reversible (to limit toxicity) inhibitors of enzymes involved in drug metabolism.

[0867] Multiple drug resistance, and hence tumor cell selection, outgrowth, and relapse, leads to morbidity and mortality in cancer patients. Libraries can be introduced into tumor cell lines (primary and cultured) that have demonstrated specific or multiple drug resistance. Target polynucleotides can then be identified which confer drug sensitivity when the cells are exposed to the drug of interest, or to drugs used in combination chemotherapy. The readout can be the onset of

apoptosis in these cells, membrane permeability changes, the release of intracellular ions and fluorescent markers. The cells in which multidrug resistance involves membrane transporters can be preloaded with fluorescent transporter substrates, and selection carried out for peptides which block the normal efflux of fluorescent drug from these cells. Libraries are particularly suited to screening for encoded peptides which reverse poorly characterized or recently discovered intracellular mechanisms of resistance or mechanisms for which few or no chemosensitizers currently exist, such as mechanisms involving LRP (lung resistance protein). This protein has been implicated in multidrug resistance in ovarian carcinoma, metastatic malignant melanoma, and acute myeloid leukemia. Particularly interesting examples include screening for target polynucleotides which reverse more than one important resistance mechanism in a single cell, which occurs in a subset of the most drug resistant cells, which are also important applications. Applications would include screening for inhibitors of both MRP (multidrug resistance related protein) and LRP for treatment of resistant cells in metastatic melanoma, for inhibitors of both p-glycoprotein and LRP in acute myeloid leukemia, and for inhibition (by any mechanism) of all three proteins for treating pan-resistant cells.

[0868] In a preferred embodiment, the present methods are useful in improving the performance of existing or developmental drugs. First pass metabolism of orally administered drugs limits their oral bioavailability, and can result in diminished efficacy as well as the need to administer more drug for a desired effect. Reversible inhibitors of enzymes involved in first pass metabolism may thus be a useful adjunct enhancing the efficacy of these drugs. First pass metabolism occurs in the liver, thus inhibitors of the corresponding catabolic enzymes may enhance the effect of the cognate drugs. Reversible inhibitors would be delivered at the same time as, or slightly before, the drug of interest. Screening of libraries in hepatocytes for inhibitors (by any mechanism, such as protein downregulation as well as a direct inhibition of activity) of particularly problematical isozymes would be of interest. These include the CYP3A4 isozymes of cytochrome P450, which are involved in the first pass metabolism of the anti-HIV drugs saquinavir and indinavir. Other

applications could include reversible inhibitors of UDP-glucuronyltransferases, sulfotransferases, N-acetyltransferases, epoxide hydrolases, and glutathione S-transferases, depending on the drug. Screens would be done in cultured hepatocytes or liver microsomes, and could involve antibodies recognizing the specific modification performed in the liver, or cocultured readout cells, if the metabolite had a different bioactivity than the untransformed drug. The enzymes modifying the drug would not necessarily have to be known, if screening was for lack of alteration of the drug.

[0869] In a preferred embodiment, the present methods are useful in immunobiology, inflammation, and allergic response applications. Selective regulation of T lymphocyte responses is a desired goal in order to modulate immune-mediated diseases in a specific manner. Libraries can be introduced into specific T cell subsets (TH1, TH2, CD4+, CD8+, and others) and the responses which characterize those subsets (cytokine generation, cytotoxicity, proliferation in response to antigen being presented by a mononuclear leukocyte, and others) modified by members of the library. Target polynucleotides can be selected which increase or diminish the known T cell subset physiologic response. This approach will be useful in any number of conditions, including: 1) autoimmune diseases where one wants to induce a tolerant state (select a peptide that inhibits T cell subset from recognizing a self-antigen bearing cell); 2) allergic diseases where one wants to decrease the stimulation of IgE producing cells (select peptide which blocks release from T cell subsets of specific B-cell stimulating cytokines which induce switch to IgE production); 3) in transplant patients where one wants to induce selective immunosuppression (select peptide that diminishes proliferative responses of host T cells to foreign antigens); 4) in lymphoproliferative states where one wants to inhibit the growth or sensitize a specific T cell tumor to chemotherapy and/or radiation; 5) in tumor surveillance where one wants to inhibit the killing of cytotoxic T cells by Fas ligand bearing tumor cells; and 5) in T cell mediated inflammatory diseases such as Rheumatoid arthritis, Connective tissue diseases (SLE), Multiple sclerosis, and inflammatory bowel disease, where one

wants to inhibit the proliferation of disease-causing T cells (promote their selective apoptosis) and the resulting selective destruction of target tissues (cartilage, connective tissue, oligodendrocytes, gut endothelial cells, respectively).

[0870] Regulation of B cell responses will permit a more selective modulation of the type and amount of immunoglobulin made and secreted by specific B cell subsets. Libraries can be inserted into B cells and target polynucleotides selected which inhibit the release and synthesis of a specific immunoglobulin. This may be useful in autoimmune diseases characterized by the overproduction of auto antibodies and the production of allergy causing antibodies, such as IgE. Target polynucleotides can also be identified which inhibit or enhance the binding of a specific immunoglobulin subclass to a specific antigen either foreign of self. Finally, target polynucleotides can be selected which inhibit the binding of a specific immunoglobulin subclass to its receptor on specific cell types.

[0871] Similarly, target polynucleotides which affect cytokine production may be selected, generally using two cell systems. For example, cytokine production from macrophages, monocytes, etc. may be evaluated. Similarly, molecules which mimic cytokines, for example erythropoetin and IL1-17, may be selected, or molecules that bind cytokines such as TNF-.alpha., before they bind their receptor.

[0872] Antigen processing by mononuclear leukocytes (ML) is an important early step in the immune system's ability to recognize and eliminate foreign proteins. Insert polynucleotides can be introduced into ML cell lines and target polynucleotides selected which alter the intracellular processing of foreign peptides and sequence of the foreign peptide that is presented to T cells by MLs on their cell surface in the context of Class II MHC. One can look for members of the library that enhance immune responses of a particular T cell subset (for example, the peptide would in fact work as a vaccine), or look for a library member that binds more tightly to MHC, thus displacing naturally occurring peptides, but nonetheless the agent would be less immunogenic (less stimulatory to a specific T cell clone). This target polynucleotide would in fact induce immune tolerance and/or diminish

immune responses to foreign proteins. This approach could be used in transplantation, autoimmune diseases, and allergic diseases.

[0873] The release of inflammatory mediators (cytokines, leukotrienes, prostaglandins, platelet activating factor, histamine, neuropeptides, and other peptide and lipid mediators) is a key element in maintaining and amplifying aberrant immune responses. Libraries can be inserted into MLs, mast cells, eosinophils, and other cells participating in a specific inflammatory response, and target polynucleotides selected which inhibit the synthesis, release and binding to the cognate receptor of each of these types of mediators.

[0874] In a preferred embodiment, the present methods are useful in biotechnology applications. Library expression in mammalian cells can also be considered for other pharmaceutical-related applications, such as modification of protein expression, protein folding, or protein secretion. One such example would be in commercial production of protein pharmaceuticals in CHO or other cells. Libraries resulting in target polynucleotides which select for an increased cell growth rate (perhaps peptides mimicking growth factors or acting as agonists of growth factor signal transduction pathways), for pathogen resistance (see previous section), for lack of sialylation or glycosylation (by blocking glycotransferases or rerouting trafficking of the protein in the cell), for allowing growth on autoclaved media, or for growth in serum free media, would all increase productivity and decrease costs in the production of protein pharmaceuticals.

[0875] Target polynucleotides encoding polypeptides or peptides displayed on the surface of circulating cells can be used as tools to identify organ, tissue, and cell specific peptide targeting sequences. Any cell introduced into the bloodstream of an animal expressing a library targeted to the cell surface can be selected for specific organ and tissue targeting. The target polynucleotide sequence identified can then be coupled to an antibody, enzyme, drug, imaging agent or substance for which organ targeting is desired.

[0876] Other target polynucleotides which may be selected using the present invention include: 1) target polynucleotides which block the activity of transcription factors, using cell lines with reporter genes; 2) target polynucleotides which block the interaction of two known proteins in cells, using the absence of normal cellular functions, the mammalian two hybrid system or fluorescence resonance energy transfer mechanisms for detection; and 3) target polynucleotides may be identified by tethering a random peptide to a protein binding region to allow interactions with molecules sterically close, i.e. within a signalling pathway, to localize the effects to a functional area of interest.

EXAMPLE 25

In vitro Depletion of T Cells Specific for Alloantigens

[0877] Human peripheral blood T lymphocytes were stimulated *in vitro* with autologous dendritic cells (DC) that were pulsed with lysate from allogeneic prostate tumor cell lines. After several cycles of restimulation with dendritic cells pulsed with tumor lysate, the CTL were tested for reactivity against tumor and the normal prostate epithelial cells from which they were derived by oncogene transformation. The NK-sensitive target, K562, was included as a control for non-specific lysis. The results in Table 13 demonstrate that these T cells were not only capable of lysing the tumor, but were equally reactive against the normal prostate cells. These data indicate that a strong T cell response is induced to tissue-specific antigens and/or alloantigens that are common to tumor and normal cells from the same donor and that are presented to allogeneic T cells by the dendritic cells. In order to isolate a minority population of tumor-specific T cells, it is therefore preferred to induce tolerance or otherwise deplete T cells reactive to normal cellular antigens of the tumor donor.

[0878] Bisindolylmaleimide VIII (Bis VIII) is one of a class of protein kinase C (PKC) inhibitors that has been shown to mediate apoptotic events. This compound

dramatically enhances T cell sensitivity to activation induced cell death (AICD) (Zhou, T., *et al.* 1999. *Nature Medicine* 5:42-48). Mouse splenic T cells activated by plate bound anti-CD3 antibody underwent dose dependent apoptosis in the presence of Bis VIII. A dose of 10 mM induced almost 100% T cell death. This property provides a method for the *in vitro* elimination of alloactivated T cells.

[0879] The protocol for T cell stimulation was modified as follows. 3×10^4 immature DCs were incubated with 1×10^5 irradiated, apoptotic *non-tumorigenic* cells in 1 ml volumes of a 24-well plate for four hours in a 37°C, 5% CO_2, humidified incubator. 5×10^6 naïve T cells (from the DC donor) were added to the wells for 24 hours. 10 mM Bis VIII was then added and incubation continued for another 24-hours. This resulted in killing of 95% of the total number of T cells, presumably those activated by normal prostate antigens and allogeneic MHC molecules. All cells were removed from the wells and washed at least 3 times to remove residual Bis VIII. The remaining tumor-specific T cells were "rescued" by restimulation for 12 days *in vitro* with fresh DCs pulsed with irradiated, apoptotic *tumor* cells. The selected cells continued to be restimulated every 12 days with tumor-pulsed DCs or irradiated tumor and autologous filler cells until their numbers were sufficient for cytokine ELISA and ^{51}Cr release assays. These $CD8^+$ CTL were specific for a shared antigen expressed by 2 prostate tumor cell lines, but did not recognize the normal prostate epithelial cells nor K562 (Table 14). The difference in recognition at an effector:target cell ratio of 5:1 is significant enough for use in antigen discovery. These CTL may be cloned and expanded to identify the shared antigen(s) being recognized.

Table 13.

Target	10:1	5:1	2.5:1
KiGT (tumor)	37	27	18
FNC267B1 (normal)	32	28	15
K562	13	6	3

Table 13. Non-tumor specific CTL induced by repeated stimulation with tumor-pulsed dendritic cells. Human peripheral blood T lymphocytes were serially stimulated by autologous dendritic cells pulsed with allogeneic tumor cell lysate (KiGT). After 3 stimulations, CTL were tested for the ability to lyse tumor cells or the normal prostate epithelial cells from which the tumor was derived by oncogene transformation. Numbers represent percent specific lysis at the indicated effector:target cell ratios in a standard 4 hour ^{51}chromium release assay.

Table 14.

Target	30:1	10:1	5:1
KiGT (tumor)	62	60	48
30Gy (tumor)	69	58	44
FNC267B1 (normal)	16	9	6
K562	2	0	0

Table 14. Prostate tumor specific CTL induced by the bis VIII tolerance method. As described in the text, a protein kinase C inhibitor, bis VIII, was employed to deplete human T cells reactive to antigens of normal prostate epithelial cells (FNC267B1). The remaining tumor-specific T cells were rescued by stimulation with autologous dendritic cells pulsed with the KiGT tumor. Specificity of the selected T cells was tested in a chromium release assay. Shared antigens expressed by a closely related tumor cell line (30Gy) are also recognized.

* * *

[0880] The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and any constructs, viruses or enzymes which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and

accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0881] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The disclosure and claims of U.S. Application No. 08/935,377, filed September 22, 1997; U.S. Application No. 60/192,586, filed March 28, 2000; U.S. Application No. 60/265,880; filed February 5, 2001; U.S. Application No. 60/271,422, filed February 27, 2001; and U.S. Application No. 60/271,424, filed February 27, 2001, are herein incorporated by reference.